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CROSS CORRELATION OF THE DORSAL ROOT
POTENTIAL AND SINGLE UNIT ACTIVITY IN THE
DORSAL HORN OF THE CAT SPINAL CORD

• ARTHUR F. JONES

1971

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CROSS CORRELATION OF THE
DORSAL ROOT POTENTIAL
AND
SINGLE UNIT ACTIVITY IN THE
DORSAL HORN OF THE CAT SPINAL CORD

by

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DEDICATION

To my wife, Pat, who
kept me relatively sane during
this,

and to Samantha-Poo,
the Cat, for putting things
in the proper perspective.

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INTRODUCTION

The gate theory of pain (39) proposes that transmission of painful sensation from the periphery to central neurons is governed by the depolarization-hyperpolarization balance at the primary afferent terminals. Depolarization of the terminals has long been monitored, after electrotonic conduction out onto the dorsal rootlets, as the Dorsal Root Potential (DRP) (14,46).

Rexed (5) has demonstrated that the dorsal horn of the spinal cord consists anatomically of layers or plates of cells stacked one on top the other in a dorsal-ventral pile. Electrophysiological studies of the horn (51,57,64,66) suggest that the anatomical divisions also carry physiological implications. Different layers of the large neurons in laminae IV-VI (5) respond to different stimuli and have different response patterns. Studies in several laboratories (55,57,64,66) have shown that cells in Rexed lamina V (physiological layer 5) discharge in response to painful cutaneous stimuli to their receptive fields. Also, Kitahata, Taub, and Sato (57) have demonstrated that

several general anesthetic agents selectively depress spontaneous activity in layer 5.

As part of a continuing study of sensory mechanisms in the dorsal horn of the cat spinal cord carried on in this laboratory, I have been recording the tonic DRP and single cell activity in characterized cells in the dorsal horn. Cross correlation between DRP and single cell activity provides information about the effectiveness of the DRP in regulating excitation of large neurons in layers 4-6.

REVIEW OF THE LITERATURE

The Dorsal Root Potential

Gotch and Horsley (32) in 1891 were the first to record electrical potentials from the spinal cord. Systematic investigation of these potentials did not occur until 1933, when Gasser and Graham (31) showed that, in response to a dorsal root afferent volley, a series of potential changes could be recorded between two electrodes placed in rostral-caudal orientation on the dorsal columns. After an initial triphasic action potential, their Dorsal Cord Potential (DCP) consisted of a negative wave (N) which they felt reflected activity in the internuncial pool, followed by a positive wave (P) which reached its peak in 20

msec. and persisted for over 100 msec. This P wave seemed to follow the time course of inhibition of flexor reflex response to a test dorsal root volley at varying times after a dorsal root conditioning volley (26).

Barron and Matthews (14) in 1938 investigated the response to a dorsal root volley by recording between two electrodes on a dorsal rootlet, one near the root entry zone and the other approximately 10 mm. distal in hopes of obtaining a simpler, more specific recording. They recorded a negative (proximal negative) Dorsal Root Potential (DRP) with a latency of 5 msec., reaching a peak at 20 msec. and slowly declining over a period of approximately 200 msec. They felt that the DRP was an index of the same process as Gasser's P wave and in fact was the same potential recorded by a more specific lead. They suggested that the DRP arose in the terminations of the dorsal root fibers themselves by a mechanism similar to that producing the negative after potential in peripheral nerves. Depolarization of both active and passive terminals was due to changing of the external medium by electrolyte flow from the still permeable portions of the active fibers. This depolarization was then electrotonically conducted out onto the dorsal root where it was recorded as the DRP. They postulated that the

potential generator for the P wave and the DRP had an inhibitory action in the cord, evidenced by this depolarization, which was due to block or depression of the effects of presynaptic excitatory impulses.

Lloyd and MacIntyre (37) in an elegant investigation of the early portion of the DRP resolved the DRP into 5 waves: the first 3 (DR I-III) bore relation to the early triphasic action potential of Gasser and Graham (31); a fourth (DR IV) seemed caused by polarization of primary afferents by neighboring fibers with some influence by secondary axons; and a fifth (DR V) which corresponded to the P wave of Gasser and Graham (31) and the DRP of Barron and Matthews (14). Lloyd and MacIntyre demonstrated that contralateral stimuli gave rise to DR I-IV at smaller stimulus strength and with shorter latency than were necessary for DR V. Ipsilateral and contralateral stimuli gave almost complete summation for DR I-IV (on a third rootlet) and almost complete occlusion for DR V, as did 2 ipsilateral stimuli. DR V was the most labile portion of the DRP to asphyxia and, when it disappeared, the antidromic spikes of the Dorsal Root Reflex (DRR) often recorded on the rising phase of V also disappeared. They agreed with Bonnet and Bremer (15) that DR V was due to activity in secondary neurons.

Frank and Puortes (29) provided convincing evidence for a presynaptic mechanism for inhibition of motoneuron

responses to dorsal root stimulation. Placing intracellular electrodes in motoneuron somata, they observed responses to stimulation of nerves from the muscles innervated by the motoneuron group and of nerves from antagonistic muscles. Inhibition from gastrocnemius nerve volleys on hamstring motoneurons was short in duration, accompanied by motoneuron hyperpolarization and decreased direct excitability. This was thought to be due to a post-synaptic mechanism. Some stimuli to hamstring nerves, however, were able to reduce the excitatory response in gastrocnemius motoneurons to gastrocnemius afferent stimulation. This inhibition was longer acting than the previous type, and was not associated with membrane hyperpolarization or changed direct excitability. The authors concluded that this inhibition was due either to a presynaptic mechanism or to an inhibitory action on dendrites sufficiently remote from the soma that potentials produced in them and conducted electronically would be missed by a recording electrode in the soma. Granit (33) has supported the latter view, but most other authors (Eccles, Wall) have accepted the former interpretation.

Eccles was able to demonstrate that volleys in Group Ia afferents from flexor muscles could produce a depression lasting approximately 200 msec. of both the monosynaptic EPSP in motoneuron somata produced by Ia afferent volleys (18) and the monosynaptic reflex

discharge (24). Eccles and his group were also able to demonstrate primary afferent depolarization of Group Ia fibers by flexor muscle group Ia conditioning volleys. Intracellular recordings from primary afferents (22) showed depolarization with the same time course as electrotonically propagated DRP's. Terminals of muscle primary afferents in motoneuron nuclei showed increased excitability to externally applied stimuli (by the method of Wall -- 45) as would be expected if they had been depolarized (23). Eccles was able to show reciprocal primary afferent depolarization (PAD) of Group Ia and Group Ib stimuli on cutaneous and Group II and Group III fibers, but little action of cutaneous, Group II and Group III stimuli on Group Ia and Group Ib terminals (20).

Using the aforementioned experiments as a basis, along with depth measurements (by a coordinate method which is at best approximate) for the field potentials associated with the DCP, Eccles (20) postulated that an interneuronal chain produced the PAD. The first cell in the pathway, or C cell, was excited monosynaptically by cutaneous, Group II and Group III impulses, but not by Group Ia or Group Ib impulses and responded with rapid repetitive firing to single cutaneous stimuli in a way that seemed correlated with the N wave of the DCP. Since the PAD has a minimum latency of 2-3 msec., Eccles postulated at least one more interneuron on the pathway --

the D cell. This cell again would respond with rapid repetitive discharges, but not monosynaptically, to cutaneous, Group II and Group III stimuli. The axons of the D cells would then terminate on the primary afferent terminals and would depolarize them by releasing a long lasting chemical transmitter. Eccles recorded several cells which he thought had the properties of D cells at depths of 1.65-2.5 mm. from the cord dorsum.

Eccles (18) concluded that the PAD acted to decrease the EPSP's by decreasing the amount of transmitter released by each presynaptic action potential.

Hagiwara and Tasaki (35) had shown in experiments on the giant synapse of the squid that decrease in the size of the presynaptic impulse to 80% of control resulted in almost complete suppression of the transynaptically evoked EPSP.

Simultaneously and independently, Wall's group was investigating the DRP elicited by stimulating cutaneous sensory afferent fibers. Wall (45) measured the antidromic dorsal root response to intramedullary stimulation by an electrode placed near what were felt to be the primary afferent terminations. He reasoned that, if the terminals became more excitable, more fibers would fire an antidromic volley in response to a given test stimulus. Recording the antidromic volley should then allow one to follow the excitability

and thus the depolarization of the terminals. This method is weighted in favor of the larger fiber responses. Wall showed that primary afferent excitability increased after a conditioning dorsal root impulse. The increase began approximately 2 msec. after the conditioning volley, reached a peak at 20 msec. and extended for over 100 msec. No change in terminal excitability was found in response to antidromic ventral root stimuli. Doses of Nembutal which were felt sufficient to block internuncial activity reduced the change by about 50%.

In 1962, Wall (46) published his hypothesis for the origin of the DRP. After recording focal potentials by a series of electrodes in the dorsal horn at times after dorsal foot stimulation, Wall computed source-sink maps for the dorsal cord. Unfortunately the locations assigned to the grid points depended on histological material in which electrodes were left in the cord and the tissue fixed. Coordinates were thus at best approximate. At 10 msec. post stimulus, sinks were found in Lissauer's tract, along the border of the lateral columns and in the ventral portion of the dorsal horn; at 20 msec., ventral sinks were decreased and the Lissauer's tract activity was moving medially; at 30 msec., when the DRP was at a maximum, the maximum sinks were in the region of the substantia gelatinosa Rolandi (SG).

In the same series of experiments, Wall used a preparation in which connections between two neighboring segments were cut one by one to show which connections were essential for DRP production. "Deafferenting" the L6 segment did not abolish the DRP recorded in it when L7 afferents were stimulated. Also, section of the dorsal columns between the two did not abolish the DRP. However, section of what was felt to be Lissauer's tract did abolish the DRP. Thus, Wall hypothesized that activity in the cells of the SG was built up by mutual bombardment. He postulated that the cells bombarded not only each other but also repetitively bombarded and depolarized the primary afferents.

Szentagothai (9) gave additional support to Wall's hypothesis by a Golgi study which showed laminae II and III or the SG (5) to form a self contained neuronal system, all axons of which either remain in the SG or return into it after an ascending or descending course in Lissauer's tract. Afferent input from both large and small dorsal root fibers were shown to form longitudinally oriented "arbors" in the area of the SG. Axons of the SG established longitudinal synaptic contacts with all terminals, dendrites, and perhaps cell bodies embedded in the SG. This demonstrated a possible anatomical substrate for presynaptic inhibition.

All of the previous studies have been investigations

of the phasic DRP produced by discrete dorsal root inputs. Mendell and Wall (40) were able to demonstrate a tonic variation in dorsal root polarization which could be shown to occlude the previously described negative DR_V . They suggested that this "tonic DRP" was caused by continuous bombardment of the spinal cord by impulses in "spontaneously active" cutaneous fibers.

Mendell and Wall also attempted to block all but C fiber input to the cord by anodal blockade of the sural nerve. Their investigations suggested that blockade of all but C fibers resulted in elimination of the negative DR_V and unmasking of a long latency, positive wave or DR_{VI} which could be eliminated by intravenous pentobarbitone in a dose of 50 mg./kg. Terminal excitability measurements by the method of Wall (45) suggested that the positive DR_{VI} was associated with decreased primary afferent terminal excitability, terminal hyperpolarization and thus increased transmitter release in response to a presynaptic impulse.

These studies of DRP characteristics led Melzack and Wall (39) to propose a new theory of pain, the "gate theory". Input from primary afferents to dorsal horn neurons, they reasoned, was governed by transmitter release from the primary afferents and thus by the depolarization-hyperpolarization balance at the primary

afferent terminals. They proposed that both large (A) and small (C) fibers in the dorsal root gave collaterals to the SG: large fibers having an excitatory input and small fibers an inhibitory input to the SG. Activity in the SG caused further excitation of SG cells and this activity caused transmitter release at axo-axonal synapses on primary afferent terminals. The afferents were depolarized resulting in inhibition of input to dorsal horn neurons. The depolarization was then recorded as the electrotonically conducted DRP. Impulses in large fibers would excite the SG and inhibit input to the dorsal horn neurons; those in small fibers would inhibit the SG and allow presynaptic impulses in primary afferents to be transmitted to dorsal horn neurons.

The production of a positive DRP by C fiber stimulation has been called into question by several investigators. Zimmermann (47) blocked A fiber conduction in the sural nerve with depolarization electrodes. C fiber stimulation in this preparation gave a negative DRP i.e. the same polarity as after A fiber stimulation. The DRP produced by C fiber stimuli could be depressed by a preceding A fiber DRP and vice versa. Franz and Iggo (30) used a low temperature thermode to selectively block A fiber conduction in the common peroneal nerve. C fiber stimulation gave DRP's with the same polarity as those obtained with A fiber stimulation. Ventral root

reflexes could also be obtained by single C fiber stimuli in unanesthetized animals and C fiber stimuli were shown to facilitate ventral root reflexes produced by A stimuli. The authors suggested that both A and C fibers could depolarize primary afferent terminals. The interactions between A and C-DRP suggested that they were both mediated by the same system.

Most of the above studies on the DRP have been done on decerebrate, spinal preparations in order to isolate the direct effects of dorsal root afferent volleys on the production of the DRP. Many authors (12,13,16,34) have also been able to demonstrate DRP production after stimulating various portions of the cerebral cortex and the brain stem. The mechanisms of production for these centrally induced DRP's are as yet unclear although their similarity to the classical DRP suggests that they may be produced in somewhat the same way. The importance of these descending DRP's in the functioning animal is at present unknown, though the possibilities suggested by a system of descending control of afferent inputs at their first central synapse are certainly exciting.

Dorsal root potentials have now been recorded for over 30 years. Thus it seems particularly surprising that the form and polarity of the classical DRP may be artifactual as has been shown by recent work of Taub, Kitehata, and Sato (unpublished data). They have shown that the bipolar recording between electrodes on a cut

distal rootlet distort the electrotonically conducted signal by a cable termination effect. Rootlet potentials caused by supramaximal A fiber stimuli to neighboring ipsilateral dorsal roots were recorded at a series of distances from the cord in cut dorsal rootlet using a muscle electrode as an indifferent. These experimenters have been able to show that the actual DRP_V is a positive, and not negative, wave of long duration. This is what would be expected if the intramedullary dorsal root terminals were being actively depolarized. These experiments call into question Wall and Mendell's (40) observation of DRP form and polarity which were based on classical "bipolar" recordings. Therefore, they also disprove assumptions about DRP polarity that form the basis for the gate theory of pain (39).

Dorsal Horn Neurons

Anatomy:

Rexed (5) was able to demonstrate, in a light microscopic study, that the dorsal horn of the cat lumbar spinal cord could be divided into six horizontal laminae. Lamina I was composed of the large marginal cells of Waldeyer; lamina II and lamina III were composed of the small cells of the substantia gelatinosa Rolandi; laminae IV, V, and VI contained the large dorsal horn neurons.

The Scheibels (6) carried out an extensive Golgi and Golgi-Cox study of terminal axonal patterns in the cat spinal cord. They showed that Lamina I had its dendritic pattern compressed in a dorso-ventral direction, following the contours of the dorsolateral and dorso-medial white matter. Lamina I received coarse afferents from the overlying white matter. Laminae II and III contained the vertically oriented "flame shaped arbors" of Szentagothai (9) composed of coarse cutaneous afferent terminals and extending for several hundred micra in a rostro-caudal direction. The apices of these arbors were capped by terminal arborizations of fine fibers emerging from Lissauer's tract. The ventral portions of the primary afferent arbors also received a less well developed capping plexus with axo-axonal synapses from the tractus cornu-commissuralis of Maric. The authors suggested that this might represent a second system for presynaptic modulation of cutaneous terminals.

The large neurons of laminae IV-VI were shown to receive complex inputs. The dendrites of Lamina IV cells were divided into dorsal, medial, and lateral groups. The dorsal dendrites were immersed in the gelatinosal neuropil; medial dendrites received input from the cornu-commissural bundle and from some fibers from the contralateral dorsal horn; lateral dendrites received input from the lateral corticospinal tract.

Lamina V dendrites extended in all directions in the transverse plane with little rostro-caudal extent. Lamina VI neurons received input on a dorsal system from primary afferents, on a lateral system from the lateral corticospinal and rubrospinal tracts, and on a medial system from contralateral primary afferents and the cornu-commissural bundle.

The Scheibels (7) were also able to study primary afferents projecting to motoneuron nuclei. They found that 30% of the afferents had collaterals which terminated directly on antagonistic muscles without intercalated neurons. They also found large numbers of afferents which projected directly to motoneurons and others directly to laminae VI-VIII without any indication of interneurons. They concluded that " it is unreasonable to hold that primary afferent depolarization depends entirely upon intercalated interneurons as a source of the presumed presynaptic depolarizing effect. We must assume instead that the presynaptic inhibition characterizing PAD may occur as a result of intrafascicular interactions within each bundle." This possibility had been pointed out by Howland et. al. (36). Katz and Schmitt (56a) has previously demonstrated that adjacent nerve fibers might interact electrically.

Belston (3,4) studied the anatomy of the dorsal horn in an electron microscopic study. No primary

afferent synapses to marginal cells were seen. He found abundant axo-dendritic and axo-axonal synapses in lamina III. In the axo-axonal synapses, the dorsal root fiber appeared to be presynaptic. Laminae IV-VI contained many axo-dendritic and axo-somatic synapses from primary afferents onto large and medium sized cells. Some axo-axonal synapses were found in which the dorsal root fiber was postsynaptic to an unknown presynaptic component.

Matsushita (2) studied the course of axonal projections from the dorsal horn neurons in a Cajal silver study. In the lumbar cord, he found numerous medium sized cells from both medial and lateral lamina V sending axons through the anterior commissure to the contralateral ventral cord. This anatomic data suggested that lamina V might be the site of origin for a spinothalamic tract.

Physiology:

Most early recordings of single unit activity in the spinal cord were done on motoneurons. Some of the first to make recordings from other units were Frank and Fuortes (28) who in 1955 published results of a series of intracellular recordings from the cord. Recordings had been made from primary afferents, ventral root fibers, interneurons and motoneurons. The authors attempted to set down some guidelines for distinguishing

signals originating in somata from those from fibers. In a later paper (53), these authors reported on their observations of spinal interneurons. They found spontaneously active units, responding to dorsal root stimuli which did not excite motoneurons and responding with bursts at frequencies of up to 200/sec. Units were not localized anatomically.

Fernandez de Molina and Gray (27) recorded slow wave "mass responses" from the dorsal horn in response to cutaneous nerve stimulation and suggested that these waves were the resultant of firing in the interneuronal pool excited by cutaneous stimuli. Maximum mass responses elicited by cutaneous afferent stimuli were substantially dorsal to those from muscle afferent stimulation.

In later work, Gray's group (49) was able to demonstrate single unit activity associated with the cutaneous mass responses. Units responding to low threshold stimuli had latencies less than 2 msec. and were dorsal to units responding to higher threshold stimuli which had latencies greater than 2 msec. Most of the low threshold cells were shown to respond to light skin touch or hair movement. Units were again located by a coordinate method which was at best approximate.

Hunt and Kuno (56) were able to record "spontaneous activity" and activity in response to dorsal root

stimulation in a random selection of dorsal horn neurons. They found considerable fluctuation in the frequency distribution of spontaneous activity in given neurons, and suggested that the spontaneous activity was thus a resultant of presynaptic inhibitory and excitatory bombardment. Unfortunately, they were working with a random selection of interneurons and did not investigate the effects of natural stimuli on their cell population.

Kolmodin and Skoglund (58) made intracellular recordings from 76 interneurons in the dorsal horn and investigated their responses to hair, pressure and nociceptive stimuli. The authors found groups of cells responsive to one and to two of the above stimuli. Only cells which were also excited by proprioceptive stimuli responded to all three. Determining cell location by leaving electrodes in situ, the authors were unable to divide the dorsal horn into discrete, localized nuclei on the basis of cell response patterns.

Much of the subsequent characterization of dorsal horn neurons was done by Wall and his group. They were able to locate cells responding to cutaneous touch in a definite lamina just ventral to the terminals of the fast sural nerve fibers (64). Cells had larger receptive fields than did primary afferents. Cell responses tended to last longer than primary afferent responses did. Unfortunately, cells were again located by fixing tissue

with electrodes in situ and measuring along them.

In 1967, Wall (66) reported that dorsal horn neurons could be divided on a physiological basis into discrete layers which seemed correlated with Rexed's anatomical laminations. Recordings were made in the lumbar cord of decerebrate cats with and without a "reversible" cold block of the cord at T13 and before and after pyramidal tract stimulation at the caudal medulla. Layer 4 cells were excited by hair movement, touch and cold. Some cells increased their firing rates with increased pressure to the skin. Cells were characterized by small receptive fields. Spinal cold block increased the frequency of spontaneous discharge without change in the size of the receptive fields. Pyramidal stimulation did not change the rate of spontaneous discharge. Mendell (60) had shown that these cells could be excited by both A and C fibers.

Wall's layer 5 cells had larger receptive fields, higher thresholds and longer latencies (by 1.5 msec.) than layer 4 did. Spinal cold block increased the frequency of spontaneous discharge. Pyramidal stimulation excited 1/3 and inhibited 2/3 of the cells.

Layer 6 cells had longer latencies than layer 5 cells by about 2 msec., and responded to cutaneous stimuli of the same magnitude necessary to stimulate layer 5. Layer 6 cells also responded to joint movement and

position. Spinal cold block increased cell response to cutaneous stimuli in some cells and decreased response to joint movement in others. All cells were depressed by Nembutal in doses of 25 mg./kg.

These results led Wall to propose a laminar organization for the dorsal horn in which cutaneous afferents excited layer 4 cells which then converged on layer 5 cells. Layer 5 axons and proprioceptive afferents converged on layer 6 cells. Pyramidal fibers synapsed on both layer 5 and layer 6 cells.

Wall (67) continued this work in a study of dorsal horn cells in spinal and intact rats. Cells in spinal rats were much like those in spinal cats with one exception: cells in rat layer 5 showed habituation to repeated stimuli; this "novelty detection" property was not observed in the cat. Layer 6 seemed to have less cutaneous input in the spinal rat than in the cat. Differences between the spinal and intact, awake preparations were more pronounced. In freely moving rats, most cells in layer 4 were not spontaneously active. More strikingly, in the intact animal, cell activity seemed affected by where the animal's attention was focused. If its attention was directed away from the receptive field of the cell in question, the cell's spontaneous discharge decreased and its threshold increased; if toward the receptive field, then the cell

was more active and its threshold was lower. Wall postulated that some of the descending presynaptic controls on afferent input previously discussed were operational in this case.

Fetz (52) investigated the effects of pyramidal tract stimulation on dorsal horn neuronal activity. Approximately 2/3 of the layer 4 cells studies were inhibited by pyramidal stimulation; about 1/3 of layer 5 cells were excited and 1/3 inhibited and about 2/3 of layer 6 cells were excited by pyramidal tract stimuli.

Pomeranz et al. (61), Hillman and Wall (55), and Selzer and Spencer (42,43) have all investigated the effects of stimulating visceral, cutaneous, and muscle afferents on dorsal horn neurons. Selzer and Spencer showed that both small visceral afferent volleys and small myelinated cutaneous volleys evoked maximal focal potentials in layer 5. They also showed that many layer 5 neurons responded to volleys in both small visceral and small cutaneous afferents thus supporting Ruch's convergence theory of referred pain (62). Pomeranz et al. also found cells in layer 5 which were excited by fine myelinated afferents from viscera, skin or muscle. These cells also had typical layer 5 cutaneous receptive fields. These authors postulated that layer 6 cells respond to large myelinated fibers from skin, layer 5 to fine myelinated fibers in skin, muscle or viscera and

layer 6 to large myelinated fibers from muscle.

The finding that layer 5 cells seem to respond specifically to what are thought to be painful stimuli form the basis for the studies of Kitahata et al. (57) on the actions of anesthetic agents and maneuvers. These investigators measured the effects of inhaled 75% N₂O - 25% O₂ and of hyperventilation (which clinically increases pain threshold) on spontaneous activity of dorsal horn neurons in decerebrate, spinal cats. N₂O and hyperventilation depressed spontaneous activity in layer 5 neurons by 49±23% and 19±9% respectively. Neither maneuver significantly affected the activity in layers 1, 4, or 6. These experiments suggest that at least some analgesic methods may act by specifically inhibiting activity at the first central neuron in the pain pathway. Kitahata et al. (57) also made electrolytic lesions at the location of cells recorded. They were able to locate all lesions. Although the method does not allow identification of individual cells, they were able to localize recording points with respect to Rexed lamina (5). Cells with response characteristics attributed to layers 1, 4, 5, and 6 were found in laminae I, IV, V, and VI respectively.

MATERIALS AND METHODS

Data is included from experiments on 7 adult male and female cats each weighing between 2.9 and 5.1 kg. Animals were initially anesthetized with 2% Halothane, 60% N₂O, and oxygen delivered by a non rebreathing system. Tracheostomy was performed and, after the animal was paralyzed with 40 mg. intrahepatic Flaxedil, the animal was artificially respired with a volume limited respirator. Ventilator tidal volume and rate were set to keep end tidal CO₂ concentration at 4 - 5%. Both common carotids were ligated. Femoral arterial and venous catheters were placed for blood pressure monitoring and intravenous Flaxedil administration (at a rate of about 15 mg./hr.) respectively. The paralyzed animal was then ventilated with room air.

The animal was then transferred to a stereotaxic apparatus and decerebrated by bilateral electrolytic lesions in the midbrain reticular formation. The vertebral column was exposed and a laminectomy was performed from L1 to L7. The vertebral column was immobilized with steel clamps. The spinal cord was transected at L1 - L2 to eliminate supraspinal control mechanisms (44) while avoiding the profound hypotension common after cervical cord section. The dura was incised under microscopic observation and pinned back to the back muscles. The

cord was flooded with mineral oil at 37° C.

Throughout the experiment, femoral arterial blood pressure, heart rate, rectal temperature and spinal cord mineral oil pool temperature were constantly monitored. Blood pressure remained above 90 mm. Hg systolic in all animals from whom data was obtained. Experience has shown that when blood pressure falls below 80 mm. Hg, dorsal horn neuronal activity is markedly disturbed when when cord circulation appears to be adequate. Temperatures were maintained between 35 and 37° C. by a heating pad and heating lamps.

For measurement of the DRP, a small rootlet from either the L6 or L7 dorsal root was carefully dissected out and cut 2-3 cm. distal to the root entry zone. The rootlet was isolated by placing Parafilm between it and the cord. The rootlet was placed over one of a pair of Ag electrodes at a distance of about 5 mm. from the cord. The other of the electrode pair was placed in contact with the paraspinal muscle and the DRP was recorded differentially between the electrodes by the method of Taub (unpublished data). The animal was uniquely grounded at another point. The DRP was amplified with Grass Model 7P5 A EEG Preamplifier ($\frac{1}{2}$ Amp Low Freq. -0.3, $\frac{1}{2}$ Amp High Freq. -75), Grass Model 7D Driver Amplifier and Textronix Model 3A74 Amplifier. Amplifier output was displayed on a Textronix type RM 565 Oscilloscope

and directly recorded on 1 track of an 8 track Philips Ana-Log 7 tape recorder.

To record dorsal horn neurons, a Transidyne "Microtrode" platinum sheathed glass microelectrode with exposed tip measuring 1 - 2 μ was inserted into the ipsilateral dorsal horn at the L7 level and lowered with a Trent Wells hydraulic microdrive. Signals from the microelectrode (against a back muscle indifferent) were recorded through a differential FET AC preamplifier, Textronix Type 1A7A differential amplifier and Textronix Type 3A74 amplifier and displayed on a Textronix Type RM 565 Oscilloscope. Cell layers were identified by spontaneous discharge pattern, modality response, and the order in which they appeared. Single unit spikes were recorded, concurrently with DRP, on another FM modulated channel of the Philips Ana-Log 7 tape recorder.

Cross correlation functions have been computed for samples of spike train and DRP on a PDP-12 computer. This method for investigating the interaction between neuronal firing patterns and slow potentials has been suggested by Gerstein (70). Computer programs for sampling and cross correlating have been selected from the "EEG Data Collection (BNI Series)" written by Dr. Grey Walter of Burden Neurological Institute. These programs were obtained from the Decus Program Library (Decus No. 12-1)

Magnetic tape recordings of spike and DRP were replayed and sampled by the computer. Samples were displayed on the computer screen and all samples containing legitimate spikes were retained on data tape for analysis. Because the minimum time per address using this program was 1 msec. and because the action potential was approximately the same length, it was necessary to replay tapes for sampling at $1/16$ of the recording speed. At this speed, spikes show their characteristic form and can be readily differentiated from noise and artifact. It is possible to analyze only 15.6 msec. (recording time) of data at one time because of limited sampling capacity. I have attempted to avoid sampling bias by retaining for analysis all samples showing definite cell action potentials. Ten to twenty-five segments of cell spike and DRP have been retained for analysis for each cell.

Cross correlation functions have been computed for each segment of cellular activity and DRP. DRP polarity was recorded so that a spike and a positive going DRP will give a positive cross correlation coefficient. Peaks in the cross correlation functions occurring at consistent time shifts have been sought. Polaroid photographs of representative samples and functions have been taken.

RESULTS

A total of twenty-six cells were isolated and characterized. Their spontaneous activity was recorded on magnetic tape concurrently with the DRP. Other cellular activity was observed but was not recorded because of difficulty isolating it from the noise level or from additional cellular discharges.

Ten layer 4 neurons were recorded. Their spontaneous activity was characterized by bursts separated by relatively quiet periods. Cells responded to light hair brushing in their receptive fields with increased firing. Pressure and pinch to the receptive field also increased cellular activity. These cells had no proprioceptive input.

Nine layer 5 neurons were recorded. Spontaneous activity consisted of bursts connected by relatively steady firing. Hair stimuli did not affect cellular activity. High threshold cutaneous stimuli such as pinch or squeeze to the receptive fields caused increased firing.

Seven layer 6 neurons were recorded. Spontaneous activity consisted of maintained bursts. Higher spontaneous firing frequencies were noted for cells in layer 6 than for those in either layer 4 or layer 5.

Cells were excited by proprioceptive input from one peripheral joint.

When more than one cell was observed as the electrode descended through the dorsal horn on one tract, cells with layer 4 characteristics and cells with layer 5 characteristics were always dorsal to those with layer 6 characteristics.

Because cells recorded in the earliest experiments were recorded at tape speeds too slow to allow further slowing necessary for sampling (as discussed above), cells could not be sampled and analyzed by our present method. Therefore, cross correlation functions were computed for sample segments from only eighteen cells: nine from layer 4; four from layer 5; and five from layer 6.

Two computer samples of spike train and DRP are shown below as Figures I and II. Both are from layer 4, cell no. 24.



Fig. I

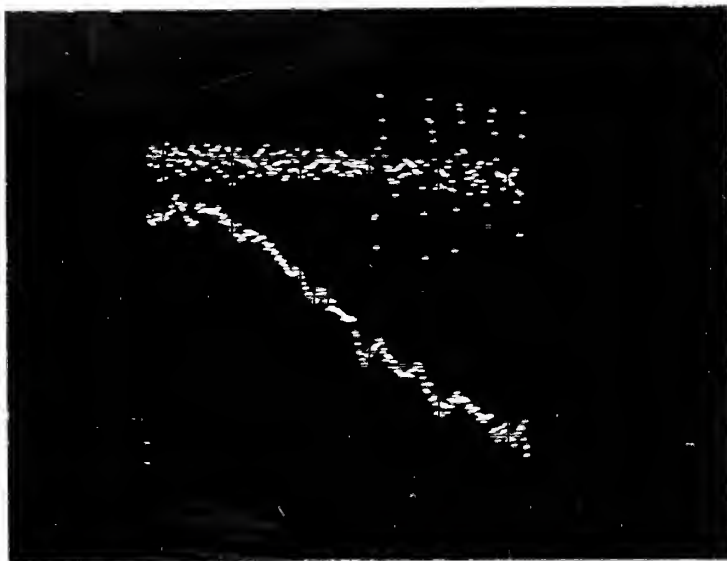


Fig. II

Figures I and II: upper trace -- spike train; lower trace -- DRP (positive voltage up). Time course is approximately 3 msec. per 1cm. oscilloscope division.

As mentioned previously, consistently appearing peaks in the cross correlation functions were sought. None were found. No cell had a consistent cross correlation function with the DRP. Implications of and possible reasons for this observation are discussed below (see DISCUSSION).

Cross correlation functions for segments I and II above respectively are shown as Figures III and IV.

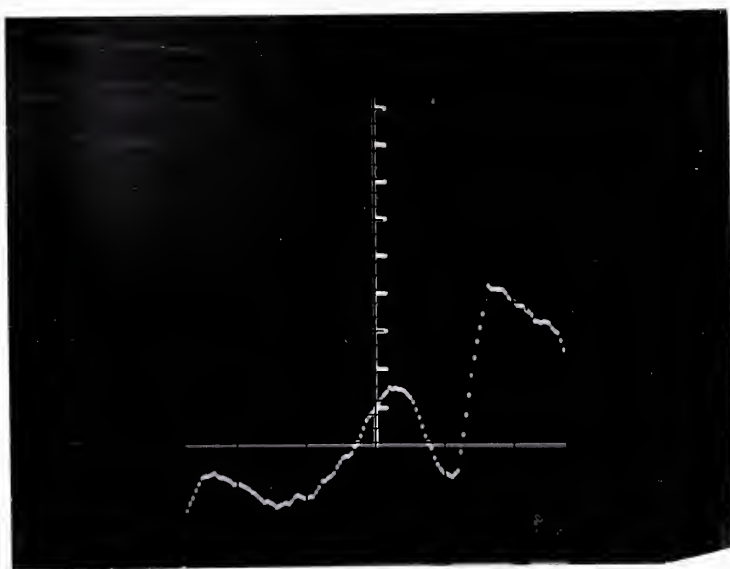


Fig. III

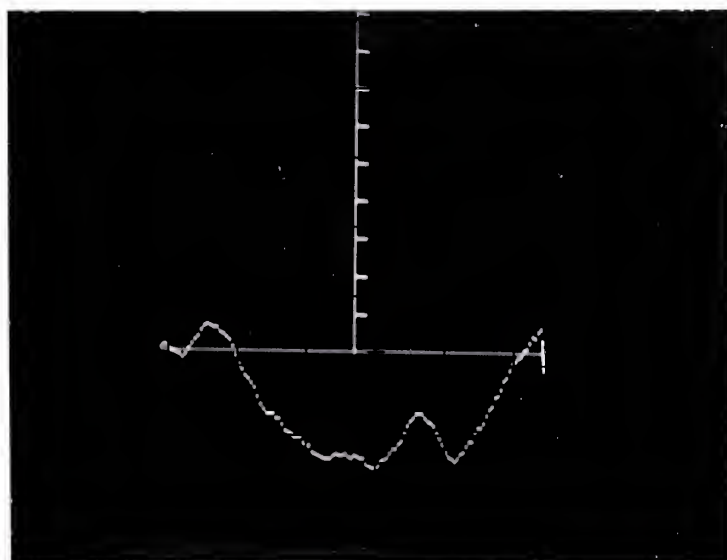


Fig. IV

Figures III and IV: x-axis --- time lead or lag, T 4 msec.; y-axis -- cross correlation coefficient, 0.1 per division.

In Figure III, the maximum positive cross correlation coefficient (+0.4) occurs at approximately +2.5 msec. Thus the curves are positively correlated with the spike trains deflections coming 2.5 msec. before the positive DRP deflection. In Figure IV, however, no positive peak occurs so the DRP and spike train in Figure II are not positively correlated within a time of ± 4 msec.

Because no consistent form was found, I have analyzed the functions in a slightly different way. I have noted the time of occurrence of the first positive peak at positive time offsets. This peak would represent the first positive time shift at which spike and DRP are positively correlated. Functions have been divided into four positive time divisions and the number of trials for which the first peak occurs in each of the time "bins" has been noted. For example, in the function shown in Figure III, the first positive peak in positive time occurs at a +0.5 msec. time offset. The first positive correlation of spike and slow wave is seen when the spike train is shifted to occur +0.5 msec. later; i.e. the spike precedes the slow wave by 0.5 msec. Numbers of spikes for each time "bin" are noted in Table I below.

In Table II, the occurrence of positive peaks in each time "bin" for each cell layer is summarized. This frequency is then compared with what would be the expected rate of occurrence if all cells were correlated

Table I

Cell Number	Cell Layer	Number of Positive Peaks	Number Trials	Location of first positive peak; Peak occurring between times noted.			
				First peak at -0.5 to +0.5 msec.	First peak at +0.5 to +1.5 msec.	First peak at +1.5 to +2.5 msec.	First peak at Greater than +2.5 msec.
7	4	3	10	2	-	-	1
8	4	4	10	1	1	-	2
9	5	3	10	-	-	2	1
10	5	5	10	1	1	1	2
11	5	5	10	2	1	-	2
12	5	2	10	1	-	-	1
13	6	3	10	-	2	-	1
14	6	2	10	1	-	1	-
15	4	2	10	-	1	-	1
16	4	2	10	-	-	2	-
17	6	6	10	2	2	-	2
18	4	1	10	-	1	-	-
19	4	6	10	1	1	1	3
20	6	3	10	-	2	1	-
22	4	7	10	-	2	2	3
24	4	9	25	1	4	4	-
26	4	6	15	1	3	1	1
27	6	6	20	1	2	2	1
Total		75	210	14	23	17	21

equally with the D&P. Significant differences from expected distribution are found in the "+0.5 msec. to +1.5 msec." bin and in the "greater than 2.5 msec." bin. Layer 5 has a lower than expected frequency of positive peaks occurring between +0.5 msec. and +1.5 msec. and a higher than expected frequency at times greater than 2.5 msec.

Table II

Layer	Positive Peaks	Total Trials	Peak Location							
			-0.5 to +0.5 msec.		+0.5 to +1.5 msec.		+1.5 to +2.5 msec.		greater than +2.5 msec.	
			Observed	Expected	Observed *	Expected	Observed	Expected	Observed **	Expected
4	40	110	6	18.7	14	32.0	10	22.7	11	28.0%
% of layer's + peaks			15.0		35.0		25.0		27.5	
5	15	40	4	18.7	2	32.0	3	22.7	6	28.0%
% of layer's + peaks			26.7		13.8		20.0		40.0	
6	20	60	4	18.7	8	32.0	8	22.7	8	28.0%
% of layer's + peaks			20.0		40.0		20.0		20.0	

* Difference from expected significant by Chi-square at p less than .01.

** Difference from expected significant by Chi-square at p less than .02.

No other distributions significantly different from expected.

DISCUSSION

The cross correlation function is a measure of correlation between two wave forms. It is computed for a number of time positions of one curve versus the other. For each time value, cross products are computed and plotted for corresponding points on the two waves and the integral of the resulting wave is plotted on the y-axis at the corresponding time on the x-axis. One curve is then shifted a small amount in time relative to the other curve and the computation repeated and another point plotted. One can then tell if two waves are indeed correlated and, if they are, at what time shift the correlation is maximum.

Present day computer facilities have made this computation practical. In my data analysis, approximately 200 cross correlation functions were computed. Each sample segment contained 254 data points and 128 time shifts were used for each function. Therefore for my experiment, the computer has made approximately $6\frac{1}{2}$ million computations.

Cross correlation functions have been suggested as a method for comparing neuron firing patterns with slow potentials by G. Gerstein (70). They are particularly useful when spontaneous activity is analyzed since in this situation one has no control over the stimuli.

The function can show a high degree of time lockedness between data samples from two sources. If a consistent high grade correlation exists, however, one is able to prove only that the patterns are consistently correlated. It is impossible to make causal inferences from the shape of the function. It is only possible to say that the function is consistent or inconsistent with a given model. For example, a high degree of correlation with a consistent time shift may exist between two cells driven by the same stimulus or between two cells, one of which excites the other.

I did not find a consistent high grade correlation between the DRP and cellular activity. One possible explanation for this is that the two patterns are not correlated. Some positive peaks in the correlation function were observed, but these might be explained as a random occurrence.

Another explanation is that the DRP must require considerable cellular activity to sustain it and I have been looking at only one cell at a time. One cell would of necessity have a limited number of synaptic contacts and its individual contribution to the DRP recorded by my method could easily be missed. In addition, it would be an over simplification to propose a mechanism for the dorsal horn's operation so rigid that the firing of one cell always signaled the firing

of cells with like properties and always produced a deflection in the DRP.

Other features of my experiment might add to difficulties in documenting correlations. Only short blocks of data from a small number of cells have been analyzed. In the future, analysis using a computer with larger storage capabilities would allow a more thorough investigation.

I have noted above that several characteristics of the positive peaks in the cross correlation functions do not appear to be consistent with a random distribution (see Table II, above). Layer 5 cells have a lower than expected incidence of correlation with the DRP for time shifts of +0.5 to +1.5 msec. and a higher than expected incidence of correlation for time shifts greater than +2.5 msec. This of course requires further documentation.

In any case all layers of the dorsal horn do not seem to have the same relationship to the DRP. Kitahata and Taub (personal communication) have also noted that the different layers have different behavior during the DRP. In response to peripheral electrical stimuli sufficient to produce a DRP, layer 4 and layer 6 cells fire a short burst during the rising phase of the DRP and then shut off. Layer 5 cells respond with a sustained burst lasting well into the course of the DRP.

Both their data and my data are inconsistent with any model which assumes comparable interaction between the DRP and layers 4, 5, and 6.

If indeed the cells of layers 4, 5, and 6 contribute to the production of the DRP, cells of layers 4 and 6 are better fitted for the early (0.5 - 1.5 msec.) activation of the wave. Layer 5, by its unexpected late (greater than 2.5 msec.) correlation with the DRP may be involved in a delayed depolarization. If layer 5 cells depolarize their own afferent set of contact, that might account for the sustained firing (and delayed inhibition) of layer 5 cells during the DRP.

The Scheibels (6) have shown that the ventral portions of the primary afferent arbors receive a capping plexus with axo-axonal synapses from the tractus cornu-commissuralis of Marie. This is in addition to the more well known dorsal capping plexus composed of fine fibers from Lissauer's tract. They have suggested that this might represent a second system for pre-synaptic modulation of cutaneous terminals. These arbors provide a possible anatomical substrate for delayed primary afferent depolarization by a feedback mechanism from layer 5 neurons.

SUMMARY

1. Twenty-six large neurons of layers 4, 5, and 6 have been isolated and characterized. Their firing pattern has been recorded concurrently with the DRP on magnetic tape.
2. Cross correlation functions have been computed for segments of DRP and spike train for eighteen cells.
3. Data inconsistent with any theory proposing comparable interaction between DRP and layers 4, 5, and 6 activity was presented and its implications were discussed.

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